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Asymmetry of Mouth Morph of a Freshwater Goby, *Rhinogobius Flumineus*

Satoko Seki[1]*, Masanori Kohda[1] and Michio Hori[2]

[1]*Department of Bio- and Geosciences, Graduate School of Science, Osaka City University, Sumiyoshi-ku, Osaka 558-8585, Japan*
[2]*Animal Ecology, Department of Zoology, Graduate School of Science, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan*

ABSTRACT—Morphological analysis of a small freshwater goby, *Rhinogobius flumineus*, showed a distinct dimorphic asymmetry in the lower jaw. The mouth of each fish opened with a slight but definite distortion toward the right or left side, depending on the individual. Right-opening mouth (dextral) fish had a right lower jaw that was more protruded than the left one, and left-opening (sinistral) fish had a more protruded left lower jaw. No fish had laterally symmetric lower jaws, indicating that the asymmetry was different from 'fluctuating asymmetry'. These fish used either the right or left side of the mouth when picking up food from the bottom, but neither dextral nor sinistral individuals used one side more frequently than the other side. The mouth asymmetry, however, was related to the stationary posture: dextral fish stayed on the bottom with the line of their bodies curved to the right more frequently than to the left, and vice versa for the sinistral fish. Genetics of the morph phenotype were investigated by observing the frequencies of morphs in F_1 under captive breeding. Mouth dimorphism seems to be determined by the Mendelian one-locus-two-alleles system, in which dextrality is dominant over sinistrality and the dominant gene acts as the lethal one when in a homozygote.

INTRODUCTION

Zoologists studying fish morphology regard the mouths of fish as being symmetrical except in the case of flatfish. Among the scale eaters in Lake Tanganyika, however, an individual's mouth opens either to the right or to the left as a result of an asymmetrical joint between the jaw and the suspensorium (Liem and Stewart, 1976; Hori, 1991, 1993). The foraging behavior in one of these scale eaters was investigated by Hori (1993). These fish dash to the prey fish from behind and tear off their scales. The fish that open their mouths to the right (dextral) always attack the left side of the prey's flank and those opening their mouths to the left (sinistral) always attack the right side. Thus, the asymmetry is clearly related to the specific prey-catching habit of this fish. More recently, Mboko *et al*. (1998) described the asymmetry of the mouth opening in a small herbivorous Tanganyikan cichlid and suggested that such asymmetric mouth openings are seen in many herbivorous cichlids in the lake. Hori (1993) indicated that asymmetric mouth morph in the scale eater, *Perissodus microlepis*, is genetically determined under the simple Mendelian one-locus-two-alleles system, in which dextrality is dominant over sinistrality. Mboko *et al*. (1998) have suggested that the asymmetry in the herbivorous cichlid is not an acquired characteristic.

The omnivorous freshwater goby, *Rhinogobius flumineus*, (Mizuno) is common in Japanese mountain streams (Mizuno, 1960; Kawanabe and Mizuno, 1989). This fish usually holds itself stationary on substrate rocks by means of a 'sucker' made of two ventral fins with the body line curving either to the right or left (Seki, pers. obs.). This fish uses either the right or left side of the mouth when picking up food (algae and insect larvae) from the bottom of the stream (Mizuno, 1960; Seki, pers. obs.). The purposes of the present study are 1) to examine the mouth morph asymmetry of this fish , 2) if the asymmetry can be detected, to examine the relations between the mouth morph and behaviors such as foraging and stationary posture and 3) to examine whether or not the asymmetry is genetic.

MATERIALS AND METHODS

A total of 135 *Rhinogobius flumineus* fish were obtained from an upper stream of the Minase River in Takatsuki City, Osaka Prefecture, Japan, in May 1995. Individuals were kept in four aquaria (30 cm × 60 cm × 45 cm deep), each containing 30–40 fish. Both sexes of this fish (4–5 cm in standard length (SL)) have sex-specific nuptial colors (Kawanabe and Mizuno, 1989) and were easily identified. Small fish without the nuptial color were regarded as young.

* Corresponding author: Tel. +81-6-6605-3170;
FAX. +81-6-6605-3172.
E-mail: satoko@sci.osaka-cu.ac.jp

Mouth morph measurements

The mouth morphs are generated by the asymmetrical joint between jaw and the suspensorium (Liem and Stewart, 1976; Mboko *et al*, 1998). But as the asymmetricity is configured by a three-dimensional distortion in the joint positions of both sides, it is not easy to determine the degree of asymmetricity with any measurements on one part.

Hori (1991) examined the asymmetry in mouth morphs of the scale eaters, in which the right and left suborbital lengths were considerably different in individuals. The degree of the deviation in *R. flumineus*, however, was much smaller, and the suborbital widths were too small to detect the difference between both sides. When the mouth of a dextral fish was open, the right side of the lower jaw protruded more than the left side, and vice versa in a sinistral fish. Thus, we measured the difference between areas covered by the arches of right and left jaws.

The jaws from formalin-fixed specimens were put in water for two days to remove the formalin and were then preserved in a 4% potassium hydroxide solution for about one week to resolve the protein. After muscle tissue was removed, the bones were preserved in 100% ethanol. We measured the areas enclosed by a line at the suture of right and left jaws, a line from the inner end of the line to the end of each jaw, and the edge line of the jaw, as indicated in Fig. 1. These areas reflect the morphological differences between right and left lower jaws. The measurements were conducted with a video micrometer (VM-30, Olympus) under a microscope. Thirty-seven dextral fish (on average 48.1 mm $\pm$ 6.1 SD in standard length (SL)) and the same number of sinistral fish (47.5 mm $\pm$ 5.1) were examined in order to determine the degree of mouth asymmetry.

Index of mouth asymmetry was calculated as:

$$(R - L) \times 100 / (R + L)$$

where R is the area covered by the arch of the right jaw and L is the area of the left jaw (Fig. 1).

Observations of fish behaviors

Feeding behaviors and stationary postures were observed in 14 dextral fish (on average 45.5 mm $\pm$ 4.1 SD in SL) and in 8 sinistral fish (45.1 mm $\pm$ 3.3). For the observations of feeding behaviors, one fish was put into a still-water aquarium 45 cm x 30 cm x 36 cm deep. Bloodworms (*Tokunagayusurika akamusi*: Chironomidae) were scattered on the bottom as prey, and the side of the mouth the fish used to pick up prey each time was recorded up to 50 times for each fish.

For the observations of stationary posture, one fish was put into a still-water aquarium. The fish stayed on the bottom for a while with the body bending to either side, then swam a short distance and stayed still again. These spells of swimming and staying still were repeated many times. Whether the fish held a right-curving or left-curving stationary posture and the duration (sec) of the posture held was recorded for more than 20 min (on average 35.2 min.$\pm$18.9 SD) for each fish.

Examination of the genetic system

The genetic system of the scale eating cichlid was of the simple Mendelian type (Hori, 1993). To examine the genetic system of the goby used in the present study, we examined the phenotype frequency of F1 in dextral (D) $\times$ sinistral (S) parents, D $\times$ D parents and S $\times$ S parents breeding in aquaria.

In natural habitats, this species reproduces in the summer (Mizuno, 1960; Kawanabe and Mizuno, 1989). Females spawn eggs under the surface of a rock, and males take care of the eggs until they hatch (Mizuno, 1960). For breeding purposes, aquaria 45 cm $\times$ 30 cm $\times$ 45 cm deep were prepared and kept at a temperature of 24°C on a 14:10 light and dark regime. In each aquarium, a 12 cm $\times$ 12 cm slate tile was put on a 4cm-thick sand bottom. A pair of fish consisting of a sexually active male and a gravid female was introduced to each aquarium. By the day after this introduction, most males had constructed a hole under the tile, and 1–3 days later the females had spawned eggs on the tile ceiling. Soon after the spawning, the tile with eggs was transferred to another 24°C aquarium in which there were no fish, since egg clutches are sometimes cannibalized by the males. The eggs on the tiles were directly exposed to water flowing down from a water filter above the aquarium until they hatched, which was usually two weeks after the spawning. The size of the hatchlings was ca. 10 mm in total length (TL).

The hatchlings were fed with brine shrimp (*Artemia* sp.) until they reached 3 cm TL, and this period usually lasted for 3 months. It was possible to identify the direction in which mouths of fish of this size opened. The fish were fixed in a 10% formalin solution, and the type of mouth-morph asymmetry (dextral or sinistral) was determined under a binocular microscope. To justify the examination, parents' names were concealed during the examination. A total of 6 broods from D $\times$ D parents, 9 from D $\times$ S parents and one from S x S parents were examined.

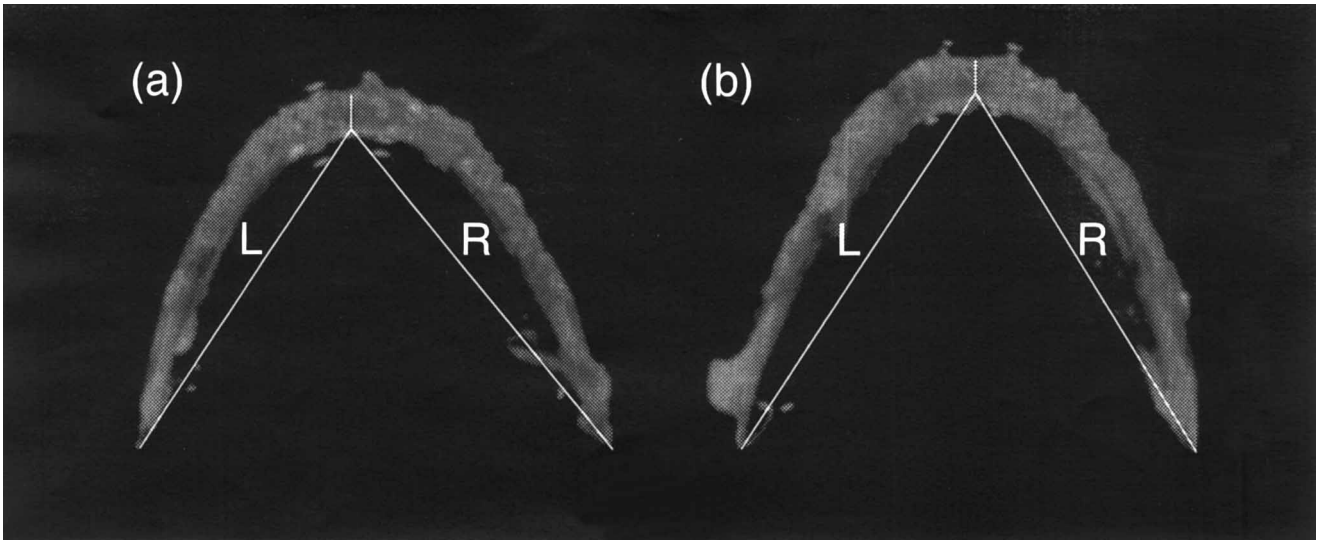

Fig. 1. Dorsal views of lower jaws of *Rhinogobius flumineus* from dextral individual **(a)** and sinistral individual **(b)**. R: an area enclosed by the arch line of right jaw and its chord (white line); L: the area of the left jaw.

RESULTS

Asymmetry in mouth morphs

The direction of the mouth opening of the 135 specimens could be categorized as rightward or leftward (Table 1). The dimorphism occurred in both sexes (binomial test: P = 0.14 in male, P=0.15 in female).

All of the 37 sinistral individuals had left lower jaws which protruded more than the right lower jaws (Fig. 1a), and dex-

Table 1. Frequency of dextral (right-opening mouth) and sinistral (left-opening) *Rhinogobius flumineus* found in 135 fish sampled. Numbers of fish are given.

	Dextral	Sinistral
Male	14	21
Female	7	13
Young	46	34
Total	67	68

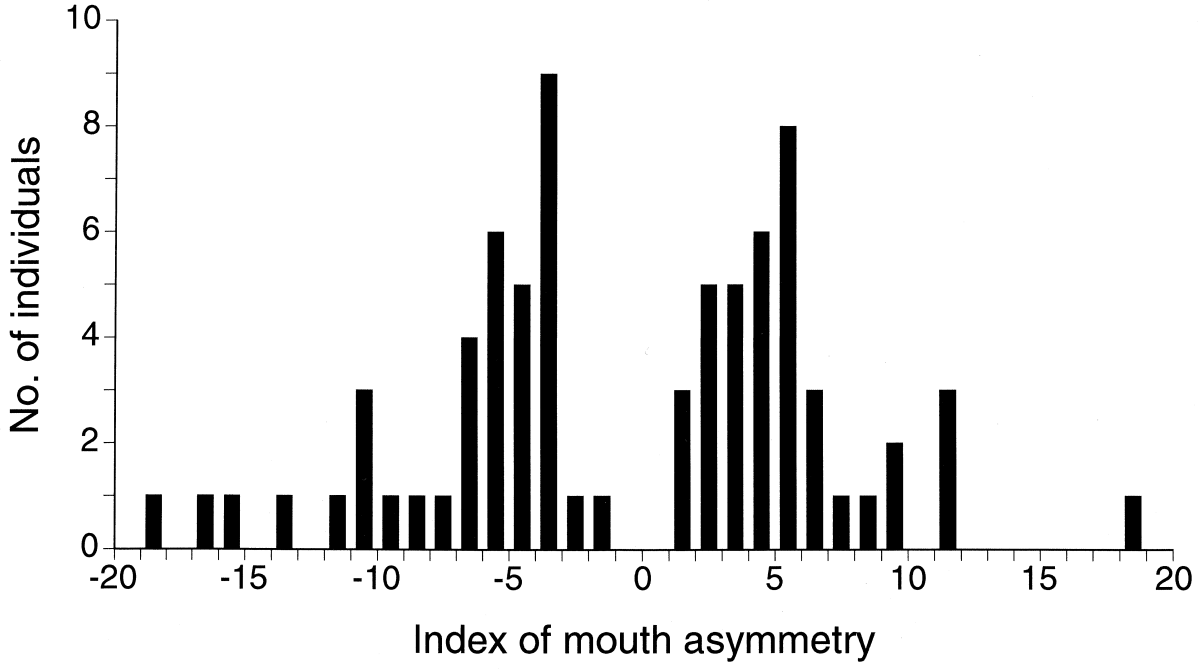

Fig. 2. The frequency of mouth asymmetry in *Rhinogobius flumineus*. Index of mouth asymmetry was calculated as: (R–L)/ (R+L) ×100. See Fig. 1 for explanation of R and L.

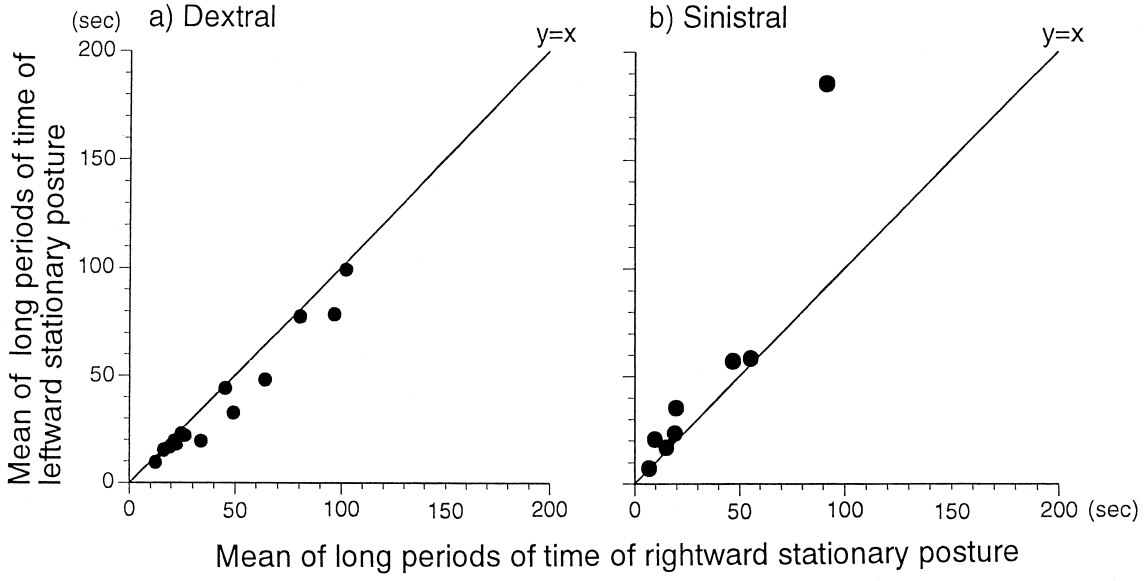

Fig. 3. Mean (in seconds) of 10 longer periods of time that dextral fish **(a)** and sinistral fish **(b)** of *Rhinogobius flumineus* remained stationary with body bending either right- or leftward.

tral individuals had protruding right lower jaws (Fig. 1b). The deviation was distinct and each type had a peak at around point 5 (Fig. 2). No fish had laterally symmetrical mouths.

Behaviors and the morph asymmetry

In most cases, the goby picked up prey (scattered bloodworms) with either one side of mouth or the other. Both dextral and sinistral fish used both sides of the mouth in foraging, and there were no significant differences in usage frequencies between right and left sides of the mouth (Fisher's exact probability test, $P > 0.1$).

In the stationary state, many dextral fish had rightward curved postures more frequently than leftward postures (12 of 18 fish) and vice versa in sinistral fish (leftward more frequently in 6 of 9 fish) (Fisher's exact probability test, P=0.036). The average length of time that rightwards and leftwards stationary postures were held by dextral fish differed significantly (Wilcoxon rank correlation test, z=2.67, P<0.01) but not for sinistral fish (z=0.98, P=0.33). In the longest 10 stays of each fish, however, dextral fish stayed in a rightward curving position for a longer period of time than a leftward curving position (Fig. 3a, z=3.30, P<0.01), and sinistral fish stayed longer in a leftward curving position (Fig. 3b, z=2.52, P<0.05).

Genetic system

Phenotype frequencies (dextral: sinistral) in F1 from parents of dextral fish were nearly 2:1 (Table 2). F1 from dextral x sinistral fish appeared in about the same number of phenotypes. F1 from parents of sinistral fish were in all sinistral offspring. The frequency in F1 from dextral parents does not correspond to the ratios expected from the simple Mendelian genetics in which dextrality is dominant over sinistrality. However, if the dominant gene is assumed to be lethal when in a homozygote, the phenotype frequencies of F1 do seem to fit the expected values (Table 2).

Table 2. Phenotype frequencies in broods of *Rhinogobius flumineus*. Expected ratios (Exp.) are those that theoretically should appear from the parents, if the genetics follow the Mendelian one-locus-two-alleles system with dextrality (D) being dominant over sinistrality (S) and the dominant gene acting as lethal when in a homozygote. The dextral parents are also assumed to be heterozygous. Figures in parentheses in uppermost line are numbers of parents.

Parents	D × D (6)	D × S (9)	S × S (1)
F1	D : S	D : S	D : S
	63 : 39 (2:1.24)	20 : 25 (1:1.25)	0 : 63 (0:1)
	26 : 23^{+} (2:1.77)	40 : 49 (1:1.23)	
	62 : 32* (2:1.03)	10 : 13 (1:1.30)	
	9 : 5**(2:1.11)	10 : 9 (1:0.90)	
	52 : 30**(2:1.15)	25 : 24 (1:0.96)	
	23 : 14**(2:1.22)	52 : 51 (1:0.98)	
		44 : 41 (1:0.93)	
		39 : 45***(1:1.15)	
		26 : 19***(1:0.73)	
Total	235 : 143 (2:1.21)	266 : 276 (1:1.04)	0 : 63 (0:1)
Exp.	2 : 1	1 : 1	0 : 1

*, **, ***: each indicates the clutches from the same pair. Difference between numbers of F1 phenotypes of each pair of D × D and D × S parents and the expected numbers of young were not significant (all $P > 0.46$, χ^2-test, except F1 with $^{+}$, P=0.16).

DISCUSSION

The deviations of an animal from perfect symmetry can be characterized by three categories: directional asymmetry, anti-symmetry and fluctuating asymmetry (Van Valen, 1962; Leary and Allendorf, 1989; Watson and Thornhill, 1994). Both directional asymmetry, such as appears in snail shells or flatfish bodies, and anti-symmetry, such as appears in chelipeds of fiddler crabs, generally result from normal development, and these individuals are clearly asymmetric. On the other hand, fluctuating asymmetry (FA) results from a disordered development, probably due to genetic homogeneity and/or to poor nutritional conditions, and FA can be found in many animals that seem to be symmetrical (Palmer and Strobeck, 1986; Watson and Thornhill, 1994). In FA, measured deviations in morphs show a normal distribution pattern with a peak at the symmetric point (Palmer and Strobeck, 1986). Detailed examination of the goby in the present study revealed that the frequency histogram of deviation in the lower jaw showed two peaks considerably apart from the symmetric point (Fig. 2). This result indicates that the mouth morph asymmetry of the goby is not a matter of FA. The same pattern in frequency of deviation was found in the scale eaters of the genus *Perissodus* (Hori, 1991) and the herbivorous cichlid (Mboko *et al.*, 1998).

Although the asymmetry of mouth morph in scale eaters was closely related to individual foraging behaviors (Hori, 1993), the mouth asymmetry of the goby, *R. flumineus*, did not correspond to feeding behaviors, as was true in the case of the herbivorous cichlid (Mboko *et al.*, 1998). However, the stationary postures of the fish were related to the mouth morphs. Although we did not examine any aspects of laterality in morphs other than that in the mouths, the correlation between mouth laterality and stationary posture suggests that the asymmetry was the result of some functional differentiation in right and left sides of body, such as a dominance in sensory abilities and/or in locomotion of one side of the body over the other.

Phenotype frequencies of F1 indicated that the dimorphism is genetic. Frequency patterns of F1 did not correspond to the ratio expected within the simple Mendelian genetic system. However, if a dominant gene is lethal in a homozygote, ratios expected under the Mendelian system seem to fit the frequencies observed in F1 of the present study. The patterns of phenotype frequencies in F1 also do not seem to fit the expected patterns in terms of quantitative genetics (Falconer, 1989). Therefore, we present the hypothesis that the mouth dimorphism is determined by the Mendelian one-locus-two-alleles system in which dextrality is dominant over sinistrality and in which the dominant gene of dextrality acts as lethal when in a homozygote. F1 from dextral x sinistral parents appeared in the same number of phenotypes (1:1) and those of F1 from dextral parents were 2:1. This suggests that there

was no individual with a homozygote of the dominant gene in the natural population, which accords with our hypothesis.

Although we did not check the mortality rate in each clutch, many eggs of certain clutches died sporadically before hatching. As the deaths occurred rather late after spawning, they could not be ascribed to non-fertilization. Neither were the deaths due to diseases such as water-mold. Unfortunately, we did not check whether or not such deaths occurred in clutches of dextral parents. We will test our hypothesis concerning the lethal gene in the near future; for example, we will check the broods from dextral parents whose mortality is 25% larger than those from the other parent combinations.

In contrast to the specialized feeding behavior of the scale eater, *P. microlepis*, the feeding behavior of the goby seem to be a generalized one, as in many fish. A mouth opening asymmetry has also been detected in a herbivorous cichlid in Lake Tanganyika (Mboko *et al.*, 1998). Although the ecological significance of the mouth-morph asymmetry in the goby, *R. flumineus*, is not clear, the discovery of the asymmetry may imply that this asymmetry will be detected in fishes from a variety of taxa.

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